California Medicine

Eosinophils and Eosinophilia

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■ *In their practice of medicine, clinicians occasionally encounter patients with eosinophilia in which the common causes have been satisfactorily ruled out. It is the purpose of this review to present some of the salient features of our present knowledge concerning the eosinophil and to suggest a method for studying eosinophilias of obscure cause.*

EOSINOPHILS ARE FORMED exclusively in the bone marrow[3] and can first be differentiated when the dark metachromatic granules in the cytoplasm of the promyelocyte begin to be replaced by granules with an intense affinity for the acid dye *eosin*.[16] These granules are large, spherical and uniform, and are sufficiently specific to identify the eosinophilic myelocyte in unstained preparations either by ordinary or phase contrast microscopy. A mature eosinophil develops from its myeloblast precursor in approximately 24 hours, as determined by marrow culture techniques.[35] It resembles the neutrophil morphologically in all respects except for the specific granules and in that its nucleus is somewhat larger and is nearly always bilobed rather than multi-lobulated.

Location

Once formed, mature eosinophils remain in the marrow an average of three or four days and constitute approximately three-fourths of the eosinophilic elements found there.[1] The marrow is the only important reserve of eosinophils in the body. From the blood stream, perhaps half leave the blood to enter the tissue during the first circulation through the body and most enter the tissue within an hour.[36] It is important to understand that the eosinophil is a cell that has its primary function in the tissue and utilizes the blood stream only for transport.[42] Fewer than 1 per cent of the total number of eosinophils in the body are to be found in the blood.[36] In normal persons, eosinophils preferentially reside in the tissues of the skin, the lung and the gastrointestinal tract.[7] Eosinophils leave these and other tissues by way of the lymphatic channels and those that are injured or senescent are selectively destroyed by the reticuloendothelial system.[1] Their total life span once they leave the marrow is eight to twelve days.[36]

Composition

Cytochemical study of the nucleus, mitochondria and cytoplasm of eosinophils has shown that they contain the same or similar substances as the neutrophil.[1] The specific granules consist of a phospholipid with a central arginine-rich protein core.[44] Considerably more work needs to be done in the cytochemistry of these granules.

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Electron microscopy has demonstrated that the granules contain a crystalloid formation[3] and microradiography shows the granules to be decidedly radiopaque.[24] Charcot-Leyden crystals are formed by the coalescence of the central electron-dense portion of the eosinophilic granules on disruption of the eosinophil.[45]

Function

The function of the eosinophil is not yet known with certainty, and one can only form conjectures from the large quantity of experimental material available. They have been shown to respond by margination, diapedesis and migration to the site of the injection of many substances.* The first injection of a suitable substance results in low-grade tissue eosinophilia, but repeated injections at intervals of three days to one week cause an arithmetic increase of both blood and tissue eosinophilia.[29] Hudson[25] pointed out the importance of considering the increased marrow reserve following the first injection in the evaluation of the eosinophilic response obtained on subsequent injections.

Speirs[41] found that repeated injection of the same or similar substances resulted in a greater eosinophilia than if widely differing substances were used. This implies a recognition on the part of the eosinopoietic system of a previously encountered eosinophilogenic substance. This and the quantitative correlation between antibody levels and eosinophil production, both in their production on exposure to an antigen and in their inhibition by irradiation and steroid therapy, led him to postulate a role for the eosinophil in antibody production. However, the temporal discordance in the appearance of antibody and eosinophils in his and others' experiments, and his failure to consider the magnitude of the marrow reserve in his measurements, have cast doubt upon his conclusions.

Aschkenasy[4] observed that eosinophils respond to the antigen and to the antigen-antibody complex but not to the host's own antibody. Litt[30] concluded on the basis of his experiments that eosinophilia was not involved in the development of antibody but that it was a response to the union of antigen and antibody. Using immunofluorescent techniques, he also demonstrated phagocytosis of antigen-antibody complexes by eosinophils, confirming Sabesin's finding with electron microscopy.[31] As to the nature of the substances possessing eosinophilotactic and eosinophilogenic capacity, all observers are in accord that they are either proteins or complex polysaccharides or combinations thereof. Constituents such as polypeptides, peptones, amino acids, carbohydrate polymers and monosaccharides have no such effect, nor do lipids.[7,11,12,29,41]

That the host proteins and polysaccharides do not cause eosinophilia is obvious and has been confirmed by autografts, injections of homologous serum proteins and autohemotherapy.[7] Speirs[41] prepared splenic extracts of mice and injected them into genetically identical mice without causing eosinophilia, whereas such preparations when injected into genetically different animals invariably did. It can therefore be concluded that the substances with eosinophilotactic and eosinophilogenic properties are proteins, complex polysaccharides or combinations thereof that are foreign to the host.

The stimulation of eosinopoiesis in the marrow and the release of eosinophils are mediated humorally, as Zaiman demonstrated with parabiotic rats.[49] Archer[2] expressed belief that the humoral substance is histamine, and other investigators[22,44,49] have reported anti-histaminic activity by the eosinophils and phagocytosis by eosinophils of mast cell granules[46] which are the main source of histamine in the body. However, some investigators have suppressed histamine release and anaphylactic symptoms by administering antihistaminic drugs before the injection of an antigen without affecting the eosinophilic response.[40]

In view of the foregoing, what is known of the function of the eosinophil can be summarized as follows. Its preferential location in the epithelial barriers implies a role in the defense of the host and its selective response to foreign protein-polysaccharide compounds further implies that this defensive role is the neutralization of these substances. This defensive role roughly parallels the production of antibody but is linked with it only insofar as both the antigen and antigen-antibody complexes constitute foreign proteins which themselves are eosinophilogenic. Eosinopoiesis, release from the marrow and eosinophilotaxis are medi-

*Reference Nos. 2, 7, 24, 43, 44, 45.

ated humorally, but histamine does not appear to be the only substance involved.

Physiologic Variation

The accepted normal blood eosinophil level in humans is from 50 to 250 per cu mm, and although no precise quantitative studies in tissues have been done, more than three or four per high-powered field would be regarded as unusual.[1] Newborns have a blood level averaging 500 to 600 per cu mm[14] which decreases to the accepted upper limit of normal within a few weeks of birth and remains within normal limits after the age of one year. This effect has been attributed to a relative adrenal insufficiency at birth. There is a slight fall in old age to the lower limits of normal, according to Buret,[14] possibly due to declining function of the eosinopoietic system. Levels in men tend to be somewhat lower than in women during adult life, and women undergo variation during their menstrual cycle, with a drop at the time of ovulation. Most observers agree on a diurnal variation in both sexes, with the levels being somewhat higher in the morning and lower later in the day. These changes are possibly linked with adrenocortical activity. Depending on their size and content, meals can cause a slight biphasic variation in the blood level also. This has been confirmed by animal study wherein eosinophils were absent from the intestinal wall in the starvation state but there was an intense infiltration when the animal was placed on a high-protein diet.[43]

Pathologic Variation

Esoinopenia results from exposure to cold, physical exertion, psychic trauma and physical injury. The mediating factor in these conditions is believed to be increased activity of the anterior pituitary-adrenal axis. Epinephrine and histamine when injected also cause eosinopenia and it is generally conceded that this is by stimulating pituitary production of ACTH. The Thorn test of adrenal capacity is based upon this principle.

The exact mode of action of the adrenal steroids in producing blood eosinopenia is not known. There is no lytic effect *in vitro* nor can increased destruction or phagocytosis be demonstrated *in vivo*. It has been been rather widely held that their action is one of blocking release from the marrow; but if this were their only effect, eosinophilic elements in the marrow would increase in proportion to the other elements during steroid therapy. No change is observed either in the number of eosinophilic elements or their mitotic activity in the marrow on initiating or maintaining corticosteroid or ACTH therapy. However, an increase in eosinophilic elements is often noted on cessation of therapy, reflected peripherally by a transient blood eosinophilia. This would suggest a suppressive effect on eosinopoiesis. However, if this were the only effect, a decreased mitosis and steady diminution in the marrow reserve of mature eosinophils should be observed during therapy, and they are not. It would appear from the above that steroids most probably neutralize the humoral substance that stimulates and coordinates both the production and the release of eosinophils from the marrow, but increased generalized dispersion in the tissues as an additional mode of action has not been ruled out.

Eosinophilia is defined by Wintrobe[48] as "the term applied to an increase in the number of eosinophilic leukocytes above the normal (250 per cu mm)," but it must be borne in mind that tissue eosinophilia is the basic phenomenon. On injection of an eosinophilogenic substance, migration of eosinophils from the blood stream to the site of injection begins almost at once. The tissue eosinophilia reaches a maximum in 12 to 24 hours and continues until the substance is neutralized. In the meantime, blood eosinopenia can be noted within an hour and the nadir is reached in two to four hours. In the marrow, a depletion of the mobile reserve can be detected four to six hours after the injection, and an increase in eosinopoiesis in approximately 12 hours. This results in blood eosinophilia in about 24 hours that continues until the injected substance is neutralized.[20] Thus the blood level may be low, normal or high with a true tissue eosinophilia, and even continue high after the tissue reaction has ceased.

The factors causing eosinophilia in the laboratory have been mentioned, and now the causes of eosinophilia from the clinical standpoint will be considered. The terms *mild* (to 10 per cent), *moderate* (10 to 30 per cent) and *marked* (over 30 per cent) will be used to give an approximate idea of the blood levels, and *transient* (a month or less) and *persistent* (more than a month) will be used to give some idea of their duration.

1. Eosinophilic leukemia has been reported to produce marked and persistent eosinophilia. Bent-

ley and coworkers[6] presented a comprehensive review of the literature on this problem. Using the definition of "a marked eosinophilia in the peripheral blood with blast cells above normal in the bone marrow and/or the peripheral blood," they were able to accept 20 cases from the literature but had to discard some 37 other reports of "eosinophilic leukemia." If one uses the definition of Bousser[9] that the case must be similar to myeloid leukemia throughout its course, but have immature eosinophils as a constant and predominant feature in the peripheral blood, then the number of acceptable cases in the literature is reduced to approximately 15. If one insists further on the demonstration of eosinophilic myeloblasts and promyelocytes in the marrow, the number is reduced to zero. Thus the disease is a strangely rare one by any acceptable definition. A pattern perhaps emerges when one notes that some of the reported cases are clearly myelogenous leukemia with a superimposed eosinophilia consisting entirely of mature eosinophils. This might represent an eosinophilic response to some abnormal proteins produced in the leukemic process or be a non-specific reflection of the abnormal marrow activity. Most of the other cases appear to be myelogenous leukemia also, but in these a variable number of immature eosinophils is found in the peripheral blood. This suggests a spectrum in myelogenous leukemia in which the eosinophil precursors may be either uninvolved, partially involved or totally involved. If this is so, then it might be better not to classify eosinophilic leukemia as a separate entity but to consider these cases as myelogenous leukemia and append a description of the extent to which the eosinophilic elements are involved.

2. Periarteritis nodosa is not ordinarily accompanied by eosinophilia, but it has been reported in up to 30 per cent of the cases in which pulmonary symptoms and pathologic changes were present. It is usually a mild, persistent eosinophilia but marked eosinophilias have been described. Some of the reported cases have not had the typical lesions but rather a form of endarteritic thickening with focal necrosis and thrombus formation. These may represent the vascular counterpart of Loffler's endocarditis parietalis fibroplastica with eosinophilia.[47] It is interesting to note that in several of the cases which could not be accepted by Bentley and coworkers as eosinophilic leukemia, the patients died in congestive heart failure after a prolonged course, and at necropsy there was myocardial infiltration by eosinophils and fibrosis with mural thrombi.[19] In all of these entities a common denominator of a blood-borne antigen causing the vascular reaction is suggested,[23] and morphologic equivalents have been described[8] that would lend support to their consideration as a group until the precise etiologic factors involved are elucidated.

3. Allergic disorders such as allergic rhinitis, asthma, angioneurotic edema, urticaria and serum sickness regularly manifest eosinophilia. This may be quite marked in the involved tissue and the cellular exudate but the blood eosinophilia is almost invariably mild. The duration of the eosinophilia corresponds to the patient's exposure to the antigen.

4. Medications, especially when given parenterally, can cause eosinophilia. The list of those reported is almost endless. This appears to be due to a constitutional characteristic of certain individuals wherein the medication is able to combine with and alter a host protein to form an antigen.[15] The eosinophilia is usually moderate but may be marked, and is transient.

5. Destructive dermatoses such as eczema, dermatitis herpetiformis, pemphigus and psoriasis can cause an eosinophilia that varies with the duration and extent of skin involvement. Any destructive lesion of an epithelial surface, such as those found in certain types of pneumonia and ulcerative colitis, for example, are capable of causing eosinophilia also, and the mechanism is believed to be the alteration of host protein in these sensitive areas. Tumors of epithelial surfaces (gastrointestinal tract, lung, skin, cervix and elsewhere) can cause a persistent, and mild to moderate eosinophilia. A neoplasm with origin elsewhere but metastasizing to a serosal surface occasionally has the same effect. Eosinophilia tends to be more frequent with tumors undergoing central necrosis.

6. Convalescence from a febrile illness often results in a mild and transient eosinophilia due to the cessation of adrenal hyperfunction and perhaps also to the production of antigen-antibody complexes.

7. Radiotherapy in repeated doses can cause a mild or moderate transient eosinophilia, apparently by altering host tissue.

8. Splenectomy can result in a mild but persistent eosinophilia starting as late as a year

after operation. The reason is not clear, but perhaps has to do with removal of the main organ of eosinophil destruction.

9. Hodgkin's disease is accompanied by eosinophilia in 5 to 10 per cent of the cases (up to 20 per cent in some series). It is most often mild and persistent, but may be marked.[33] It is more frequently found in abdominal Hodgkin's disease where ulceration of Peyer's patches has been demonstrated and thus it might possibly be due to the decreased integrity of the intestinal epithelial barriers in excluding intestinal contents. Production of an abnormal protein is another possible explanation.

10. Loffler's syndrome has stood the test of time as a workable clinical classification, but in using the term one must be careful to heed the original definition.[32] The eosinophilia is mild and transient. Crofton,[13] created a classification of pulmonary diseases accompanied by eosinophilia that has proven useful in cases that do not fit Loffler's definition, although the categories are grouped according to severity, which is not necessarily concordant with etiologic factors.

Approximately 50 per cent of patients with Loffler's syndrome have a history of allergic diathesis in themselves, in collateral relatives, antecedents or progeny, and it is now the consensus that the syndrome is produced by the pulmonary migration of intestinal parasites (especially Ascaris) in presensitized or predisposed persons.[13]

11. Tropical eosinophilia[17] and the Meyers-Kouwenaar syndrome [34] are believed to be caused by zoonotic filarids.[10] In both, the eosinophilia is marked and persistent.

12. Cutaneous larva migrans results from skin contact with soil containing larvae of certain zoonotic parasites.[21] The eosinophilia tends to be very mild but persistent.

13. Visceral larva migrans is caused by migration throughout the human body by larvae of zoonotic helminths.[5,38] In this category should be included gnathostomiasis, sparganosis and the eosinophilic meningoencephalitis first reported in Tahiti.[45] Eosinophilia tends to be moderate to marked and persistent. It is now believed that the entity "familial eosinophilia" is actually visceral larva migrans, with several members of a family being infected from a common environmental source.

14. Parasites for which the human is accepted as an intermediate or definitive host cause the majority of the cases of mild eosinophilia encountered clinically.[26] The degree varies with the nature of the parasite, its location or migration in the host, and the intensity of the infection. The duration varies with the exposure to the parasite and its products. Protozoa, like viruses, bacteria and fungi, do not cause eosinophilia except secondarily through epithelial destruction.

In the metazoa, the mature cestode in the gut does not usually cause eosinophilia, but if great numbers are attached to the mucosa as with *Hymenolepis nana,* there may be mild eosinophilia. When man is the intermediate host as in echinococcosis and cysticercosis, onchospheric migration and cyst rupture with extravasation of its contents can cause a moderate to marked but transient eosinophilia.

Among the trematodes, those which reside in the intestinal lumen do not usually cause eosinophilia; those in the biliary tract usually cause a mild, persistent eosinophilia which may become moderate during migration and oviposition; those inhabiting the veins of the intestine and bladder cause a persistent mild to moderate eosinophilia which may be marked during metacercarial migration and oviposition. *Paragonimus westermanni,* which resides in the lungs, causes a mild to moderate eosinophilia varying with the intensity of the infection.[18]

In nematodes, those living free in the intestinal lumen or superficially attached to the mucosa do not cause eosinophilia. However during migration of the larvae through the lungs, a moderate to marked but transient eosinophilia results. Those which invade, live in and deposit larvae in the intestinal wall and other tissue cause a moderate to marked eosinophilia. Filarids cause a variable eosinophilia depending upon the characteristics of the species. *Dracunculus medinensis* is usually sheathed in a fibrous capsule and rarely causes eosinophilia. The adults of *Onchocerca volvulus* also tend to remain in fibrous onchocercomas but discharge microfilariae, and may therefore cause a persistent eosinophilia of mild proportions. The adult *Loa loa* migrates in the subcutaneous tissues and also produces microfilariae; thus the resulting eosinophilia is usually moderate. Among the arthropod ectoparasites, only *Sarcoptes scabei* and *Tunga penetrans* invade the skin to any extent, and with them eosino-

philia is rare and mild when it does occur. Myiasis caused by the larvae of many cyclorraphous flies is rarely accompanied by eosinophilia, as their migration is minimal and the larvae tend to become enclosed in fibrous capsules.

Diagnosis

A systematic approach is required in the diagnosis of eosinophilias, because the usual investigation may fail to lead to a diagnosis in as many as 60 per cent of the cases, and the patients therefore do not receive specific treatment.[27] The first step is to obtain one or more absolute eosinophil counts to make sure that eosinophilia actually exists, to ascertain the level of eosinophilia and to establish a baseline against which to measure the progress of the disease and the effect of therapy. As eosinophils tend to adhere to the glass edge and congregate at the tail of the smear, a report based on the percentage encountered in the smear is subject to laboratory error of up to 20 per cent.[43] Evaluation should then proceed as follows:

1. A complete history must be taken, with emphasis upon allergic diathesis; geographic areas visited during the past year; contact with animals and their excreta; geophagia or pica; eating meat, fish, shell fish or aquatic plants raw or poorly cooked; skin contact with the ground and with fresh water streams or lakes; medications taken within past month; and recent febrile illnesses, radiation or splenectomy. In the system review particular attention should be paid to the skin and the gastrointestinal and respiratory tracts.

2. The physical examination must include a careful examination of the skin with scrapings of wheals or other lesions for eosinophils; palpation for nodes and hepatic or splenic enlargement; examination of the nasal mucous membranes with a smear for eosinophils if the membranes appear to be involved; careful examination of the chest and abdomen for evidence of respiratory or gastrointestinal disease; and a rectal-sigmoidoscopic examination. Women should have a pelvic examination with a Papanicolaou smear.

3. Absolute eosinophil counts of the peripheral blood should be done periodically, and blood smears should be examined at the same time for blood-borne parasites and immature eosinophils. If immature cells are found, a bone marrow aspiration is indicated.

4. If respiratory symptoms are present, at least three specimans of sputum should be examined for malignant cells, ova, parasites, eosinophils and Charcot-Leyden crystals. Bronchoscopy with aspiration and other specialized procedures can be considered.

5. An x-ray film of the chest, an upper gastrointestinal series and studies with barium enema should be routine in all cases.

6. At least three fresh stools should be examined by a competent experienced laboratory technician for ova and parasites, and at least one specimen should be studied with a concentration technique. If strongyloidiasis is suspected, duodenal intubation and aspiration should be considered. If schistosomiasis is a possibility, the urine sediment must be examined carefully. The stools should be routinely examined also for occult blood, eosinophils and Charcot-Leyden crystals.

7. A panel of liver function tests is indicated.

8. Biopsy of any suspicious skin lesion is required. If such lesion is present, then biopsy of a specimen of the skin, including subcutaneous tissue and muscle, taken at random should be done.

9. Biopsy of one or more specimens of the liver is indicated if there is hepatomegaly, liver tenderness or alteration of the liver function tests. Specimens must be divided into three portions and examined as fresh press preparations, after pepsin digestion, and as serial sections of fixed tissue.

10. Biopsy of enlarged or tender nodes must be done, using the same examination techniques as for the liver.

11. Skin tests using parasite antigens are of value only if negative, due to the persistence of positivity and the frequency of cross reactions. Serologic methods of value in some parasitic diseases have been developed and serum can be sent to health centers and institutions that perform these tests. Increasing reliability and specificity is being obtained by investigators active in this field.[28,37]

By following the rules set forth above, the author has been able to make specific diagnosis in most cases. However, if the eosinophilia remains etiologically obscure despite this investigation, continued observation during careful therapeutic trials is the only remaining resource.

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